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(54) **Title:** FELINE IMMUNODEFICIENCY VIRUS ISOLATE NCSU 1

(57) **Abstract**

Disclosed is an isolated and purified feline immunodeficiency virus (FIV) culture having the identifying characteristics of FIV isolate $NCSU_1$. A biologically pure culture of host cells containing an FIV having the identifying characteristics of FIV isolate $NCSU_1$ is also disclosed, along with isolated and purified DNA coding for (a) an FIV having the identifying characteristics of FIV isolate $NCSU_1$, or (b) an antigenic fragment of an FIV having the identifying characteristics of FIV isolate $NCSU_1$. Various vaccine formulations containing active agents derived from the foregoing FIV virus, DNA encoding the virus, and DNA encoding antigenic fragments of the virus are also disclosed herein. Also disclosed are immunodeficient mice containing feline tissue, which feline tissue is capable of infection with a feline immunodeficiency virus such as (but not limited to) FIV isolate $NCSU_1$.

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FELINE IMMUNODEFICIENCY VIRUS ISOLATE NCSU 1

This invention was made with government support under Public Health Service grant CA-43676 from the National Cancer Institute. The government may have certain rights to this invention.

Field of the Invention

This invention concerns a unique isolate of Feline Immunodeficiency Virus which is highly infectious in vivo and produces a rapid inversion of the $CD4^+$:$CD8^+$ receptor ratio in infected subjects.

Background of the Invention

Feline immunodeficiency virus (FIV), a lentivirus of cats, is associated with feline acquired immunodeficiency syndrome (AIDS). *See* N. Pedersen et al., *Science* **235**: 790 (1987). Disorders associated with FIV infection include chronic gingivitis/stomatitis, chronic upper respiratory infections, chronic enteritis, and recurrent ocular disease. *See* R. English et al., *J. Am. Vet. Med. Assoc.* **196:** 1116 (1990); N. Pedersen et al., *Vet. Immunol. Immunopathol.* **21**: 111 (1989); J. Yamamoto et al., *J. Am. Vet. Med. Assoc.* **194:** 213 (1989). What is known to date of the pathogenesis of FIV infection suggests that it is a valuable animal model for human immunodeficiency virus-1 (HIV-1)-induced AIDS. HIV-1 and FIV belong to the lentivirus subfamily of retroviruses, have similar morphology, protein composition, and Mg^{2+}-dependency of their reverse transcriptases (RT). *See* N. Pedersen et al., *Science*

235: 790 (1987); N. Pedersen et al., *Vet. Immunol. Immunopathol.* **21**: 111 (1989). They both display tropism for T lymphocytes and monocytes and are capable of inducing these cells to form syncytia. *See* D. Brunner and N. Pedersen, *J. Virol.* **63**: 5483 (1989); M. Gardner and P. Luciw, *FASEB Journal* **3**: 2593 (1989). HIV-1 displays a particular tropism for $CD4^+$ lymphocytes, which leads to their gradual depletion and an inversion of the $CD4^+$:$CD8^+$ ratio. *See* A. Dalgleish et al., *Nature* **312**: 763 (1984). The pathogenesis of HIV-1 infection has been attributed to virus-induced reduction of $CD4^+$ lymphocyte numbers and functions, resulting in decreased immune responsiveness and subsequent severe secondary infections. *See* M. McChesney and M. Oldstone, *Ad. Immunol.* **45**: 335 (1989).

Yamamoto et al. recently studied the early events in the pathogenesis of FIV in kittens. *See* J. Yamamoto et al., *Am. J. Vet. Res.* **49**: 1246 (1988). These kittens developed an acute infection syndrome similar to that seen in HIV-1, including low grade fever and transient generalized lymphadenopathy. More recent studies by Ackley et al., *J. Virol.* **64**: 5652 (1990), utilized monoclonal antibodies directed against feline $CD4^+$ and $CD8^+$ homologues and Pan T cells to analyze lymphocyte profiles in SPF cats experimentally infected with FIV. These authors reported that a significant inversion of the $CD4^+$:$CD8^+$ ratios occurred only in cats infected for 18 months or more. The inversion was associated with a decrease in absolute number of $CD4^+$ cells and an increase in $CD8^+$ cells.

We have recently utilized a panel of monoclonal antibodies specific for feline T cell subsets (M. Tompkins et al., *Vet. Immunol. Immunopathol.* **26**: 305 (1990)) to analyze T cell numbers and profiles in cats naturally infected with FIV. *See* C. Novotney et al., *AIDS* **4**: 1213 (1990). Similar to the observation of Ackley et al. *supra*, cats naturally infected with FIV

have an inverted $CD4^+$:$CD8^+$ ratio characterized by a selective reduction in $CD4^+$ cells. The present invention arose from our continuing efforts to better understand the early events in FIV infections.

Summary of the Invention

A first aspect of the present invention is isolated feline immunodeficiency virus (FIV) having the identifying characteristics of FIV isolate $NCSU_1$.

A second aspect of the present invention is a biologically pure culture of host cells containing a FIV having the identifying characteristics of FIV isolate $NCSU_1$.

A third aspect of the present invention is isolated DNA coding for (a) an FIV having the identifying characteristics of FIV isolate $NCSU_1$, or (b) an antigenic fragment of an FIV having the identifying characteristics of FIV isolate $NCSU_1$.

Various vaccine formulations containing active agents derived from the foregoing FIV virus, DNA encoding the virus, and DNA encoding antigenic fragments of the virus are also disclosed herein.

A further aspect of the present invention is a host cell containing the recombinant DNA sequence as given above and which expresses the encoded polypeptide or antigenic fragment thereof.

Also disclosed are immunodeficient mice containing feline tissue, which feline tissue is capable of infection with a feline immunodeficiency virus, with the FIV preferably (but not necessarily) being FIV isolate $NCSU_1$.

Brief Description of the Drawings

Figure 1 shows hybridization analysis of PCR amplified FIV DNA from PBMC of $NCSU_1$ virus inoculum source cats. Lanes 1-15 represent serial 10 fold dilutions

(beginning at 1 x 10^6 PBMC) from a naturally infected cat (TOM, lanes 1-5) and 2 SPF cats inoculated with PBMC from TOM (LOU, lanes 6-10; JJ, lanes 11-15). Lane 16 is PCR amplified control DNA from FIV-infected CrFK cells. TOM and JJ yielded provirus in as few as 1 x 10^3 PBMC and provirus was amplified in as few as 1 x 10^2 PBMC from LOU.

Figure 2 shows Mg^{2+}- (FIV) and Mn^{2+}- (FeLV, FeSFV) dependent RT activity in co-culture supernatants of PBMC from a cat infected with FIV 6 weeks previously. Numbers represent the mean of quadruplicate samples.

Figure 3 shows changes in $CD4^+$ and $CD8^+$ cell numbers and the $CD4^+$:$CD8^+$ ratio during FIV infection. Cell numbers were determined by multiplying the percent positive $CD4^+$ or $CD8^+$ cells, determined by flow cytometric analysis, by the total lymphocyte count from a CBC (drawn at the same time as the sample for flow cytometry). (A) Cell numbers and ratio of a representative FIV-infected cat (MID). (B) Mean cell numbers and ratio of all 6 FIV-infected cats. There is a significant relationship between T cell numbers ($CD4^+$: $p = 0.0005$; $CD8^+$: $p = 0.0271$) and time post infection.

Figure 4 shows the mean $CD4^+$ and $CD8^+$ cell numbers and $CD4^+$:$CD8^+$ ratio of 3 normal, random source cats that had blood samples collected at the same time as the infected cats. There is no significant relationship between T cell numbers and week of sampling.

Figure 5 shows the mean $CD4^+$:$CD8^+$ ratios of 6 FIV-infected cats and 3 control cats. The bars indicate 1 standard deviation. There is no significant difference in $CD4^+$:$CD8^+$ ratios of the two cat populations prior to infection (0 time), but at 4 weeks p.i. and there after, there is a significant difference in the ratios ($p < 0.0001$).

Figure 6 shows the mean $CD4^+$ and $CD8^+$ cell numbers and $CD4^+$:$CD8^+$ ratio of 4 mock-infected cats. There is no significant relationship between the T cell numbers and week post inoculation.

Detailed Description of the Invention

Amino acid sequences disclosed herein are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence. Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code in accordance with 37 C.F.R. §1.822 and established usage. *See, e.g.* PatentIn User Manual, 99-102 (Nov. 1990) (U.S. Patent and Trademark Office, Office of the Assistant Commissioner for Patents, Washington D.C. 20231); U.S. Patent No. 4,871,670 to Hudson et al. at Col. 3, lines 20-43 (applicants specifically intend that the disclosure of this and all patent references cited herein are to be incorporated herein by reference).

Aspects of the present invention are achieved by a virus having the identifying characteristics of the deposit designated Feline Immunodeficiency Virus (FIV-$NCSU_1$), made in accordance with the provisions of the Budapest Treaty on July 23, 1991, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 USA, and assigned ATCC Number VR2333.

A. Identification of Antigenic Fragments

Antigenic fragments of the present invention are peptides which contain at least one epitope (antibody binding site) which binds antibodies which bind to the FIV isolate of the present invention. The antigenic fragments are preferably capable of inducing an immune response when administered to a feline subject, as discussed in greater detail below. In addition, the antigenic fragments preferably bind antibodies which do not bind to prior FIV isolates. DNA encoding such antigenic fragments may be used to transform host cells

to thereby produce such antigenic fragments, as explained in greater detail below. Antigenic fragments may be identified by a variety of means. A protein from FIV isolate $NCSU_1$, such as the envelope protein, may be fragmented with a protease, and the fragments tested to determine whether or not various ones react with antiserum against the protein. *See, e.g.*, J. Robinson et al., *Mol. Cell Biochem.* **21**, 23-32 (1978). Another technique is to synthesize peptides which are fragments of the entire protein, and determine whether the individual fragments are recognized by neutralizing antibodies against the protein. *See, e.g.*, J. Gerin et al., *in Vaccines 85: Molecular and Chemical Basis of Resistance to Parasitic, Bacterial and Viral Diseases*, 235-239 (Lerner et al., eds. 1985). Still another method useful for obtaining immunogenic fragments of a protein is by isolation and identification of monoclonal escape mutants. In this strategy, FIV is produced in the presence of a monoclonal antibody to the virus. The only virus which can grow under these conditions are those with a mutation in the nucleotide sequence which codes for an epitope to which the monoclonal antibody binds. A mutant virus which grows under these conditions is referred to as the "monoclonal escape mutant." The monoclonal escape mutant is then sequenced and the mutant sequence compared with the nucleotide sequence of FIV isolate $NCSU_1$ to find the specific location of the mutation. The mutation is located in a region which codes for a protective epitope, or an "immunogenic fragment." *See, e.g.*, J. Lopez et al., Location of a Highly Conserved Neutralizing Epitope in the F Glycoprotein of Human Respiratory Syncytial Virus, *J. Virol.* **64**, 927 (1990).

B. Genetic Engineering Techniques

The production of DNA, vectors, transformed host cells, FIV virus, proteins, and protein fragments of

the present invention by genetic engineering techniques can be carried out in accordance with methods known in the art. See, e.g., U.S. Patent No. 4,761,371 to Bell et al. at Col. 6 line 3 to Col. 9 line 65; U.S. Patent No. 4,877,729 to Clark et al. at Col. 4 line 38 to Col. 7 line 6; U.S. Patent No. 4,912,038 to Schilling at Col. 3 line 26 to Col. 14 line 12; and U.S. Patent No. 4,879,224 to Wallner at Col. 6 line 8 to Col. 8 line 59.

Vectors are replicable DNA constructs used to either amplify or express DNA of the present invention. An expression vector is a replicable DNA construct in which DNA of the present invention is operably linked to control sequences capable of expressing that DNA in a suitable host. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Suitable vectors include plasmids, viruses (e.g., vaccinia virus, adenovirus, baculovirus, cytomegalovirus), phage, and integratable DNA fragments (i.e., fragments integratable into the host genome by recombination).

DNA regions are operably linked or operably associated when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation.

Transformed host cells are cells which have been transformed or transfected with vectors as described above. Transformed host cells ordinarily express the DNA of the present invention. Suitable host cells include prokaryote, yeast or higher eukaryotic cells such as mammalian cells and insect cells.

Prokaryote host cells include gram negative or gram positive organisms, for example *Escherichia coli* (*E. coli*) or Bacilli. Exemplary host cells are E. coli W3110 (ATCC 27,325), E. coli B, E. coli X1776 (ATCC 31,537), E. coli 294 (ATCC 31,446). A broad variety of suitable prokaryotic and microbial vectors are available. E. coli is typically transformed using pBR322. Promoters most commonly used in recombinant microbial expression vectors include the β-lactamase (penicillinase) and lactose promoter systems (Chang et al., Nature 275, 615 (1978); and Goeddel et al., Nature 281, 544 (1979)), a tryptophan (trp) promoter system (Goeddel et al., Nucleic Acids Res. 8, 4057 (1980) and EPO App. Publ. No. 36,776) and the tac promoter (H. De Boer et al., Proc. Natl. Acad. Sci. USA 80, 21 (1983)). The promoter and Shine-Dalgarno sequence are operably linked to the DNA of the invention, i.e., they are positioned so as to promote transcription of messenger RNA from the DNA.

Eukaryotic microbes such as yeast cultures may also be transformed with vectors of the present invention. see, e.g., U.S. Patent No. 4,745,057. *Saccharomyces cerevisiae* is the most commonly used yeast, although other yeast may also be used. Yeast vectors may contain an origin of replication from the 2 micron yeast plasmid or an autonomously replicating sequence (ARS), a promoter, an axl oncogene, sequences for polyadenylation and transcription termination, and a selection gene. An exemplary plasmid is YRp7, (Stinchcomb et al., Nature 282, 39 (1979); Kingsman et al., Gene 7, 141 (1979); Tschemper et al., Gene 10, 157 (1980)). Suitable promoting sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., J. Biol. Chem. 255, 2073 (1980) or other glycolytic enzymes (Hess et al., J. Adv. Enzyme Reg. 7, 149 (1968); and Holland et al., Biochemistry 17, 4900 (1978)).

Host cells such as insect cells (e.g., cultured *Spodoptera frugiperda* cells) and expression vectors such as the baculovirus expression vector (e.g., vectors derived from *Autographa californica* MNPV, *Trichoplusia ni* MNPV, *Rachiplusia ou* MNPV, or *Galleria ou* MNPV) may be employed in carrying out the present invention, as described in U.S. Patents Nos. 4,745,051 and 4,879,236 to Smith et al. In general, a baculovirus expression vector comprises a baculovirus genome containing the gene to be expressed inserted into the polyhedrin gene at a position ranging from the polyhedrin transcriptional start signal to the ATG start site and under the transcriptional control of a baculovirus polyhedrin promoter.

Examples of useful mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and WI138, BHK, COS-7, CV, and MDCK cell lines. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells are often provided by viral sources. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and Simian Virus 40 (SV40). See, e.g., U.S. Patent No. 4,599,308. An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g. Polyoma, Adenovirus, VSV, or BPV) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient. Rather than using vectors which contain viral origins of replication, one can transform mammalian cells by the method of cotransformation with a selectable marker and DNA of the present invention, as described in U.S. Pat. No. 4,399,216.

C. **Vaccines and Vaccine Formulations.**

The present invention provides for a variety of different vaccines useful for protecting feline species

against FIV. Examples include live attenuated FIV isolate $NCSU_1$ virus, fixed whole virus, host cells which express virus antigen on the surface thereof (with the cells optionally fixed), preparations of virus fragments, purified proteins, antigenic fragments of proteins, and antigenic peptides which are derivatives of the antigenic fragments (as discussed in detail below). These various compounds and mixtures are generically referred to herein as active agents.

Live attenuated FIV isolate $NCSU_1$ virus is made by serial passage of the virus in tissue culture or genetically altered by recombinant techniques, in accordance with known procedures. Fixed virus is made by contacting live virus (attenuated or unattenuated) to a suitable fixative, such as formalin.

Preparations of viral fragments are made by lysing host cells, such as *E. coli* cells, transformed with a vector encoding the FIV isolate of the present invention or a portion thereof. For example, the vector may encode an FIV isolate which produces hollow virus particles which are antigenic. The lysate may be used in crude form, partially purified, or a particular viral protein (or antigenic fragment thereof) such as the envelope protein purified to homogeneity, and used as an active agent for a vaccine against FIV.

Host cells such as yeast cells may be transformed with vectors of the present invention capable of expressing FIV proteins, or antigenic fragments thereof, on the surface of the host cells, and the transformed host cells used as an active vaccine agent per se or fixed (e.g., with formalin) and used as an active agent.

Antigenic peptides are selected from the group consisting of antigenic fragments of FIV isolate $NCSU_1$ proteins, such as the envelope protein, and the antigenic equivalents thereof (i.e., analogs or derivatives). Antigenic peptides may be chemically synthesized or

produced by recombinant techniques. The antigenic fragments are preferably not more than 20 amino acid residues in length, and are more preferably not more than 10 amino acid residues in length. The antigenic equivalents are selected from the group consisting of: (a) modified peptides comprising the aforesaid antigenic fragments modified by the inclusion of one or more changes to the amino acid sequence thereof; and (b) longer peptides which incorporate the sequence of the aforesaid fragments or the aforesaid modified peptides and which have (i) up to four extra amino acid residues attached to the C-terminal end thereof, (ii) up to four extra amino acid residues attached to the N-terminal end thereof, or (iii) up to four extra amino acid residues attached to the C-terminal end thereof and up to four extra amino acid residues attached to the N-terminal end thereof.

The term "antigenic equivalents," as used herein, refers to proteins or peptides which bind to an antibody which binds to the protein or peptide with which equivalency is sought to be established. Antibodies which are used to select such antigenic equivalents are referred to as "selection antibodies" herein. Preferred selection antibodies are monoclonal antibodies which bind to FIV isolate $NCSU_1$, but not to prior isolates of FIV such as the Petaluma strain isolated by N. Pedersen.

One or more amino acids of an antigenic peptide sequence may be replaced by one or more other amino acids which does not affect the antigenicity of that sequence. Such changes can be guided by known similarities between amino acids in physical features such as charge density, hydrophobicity/hydrophilicity, size and configuration. For example, Thr may be replaced by Ser and vice versa, Asp may be Replaced by Glu and vice versa, and Leu may be replaced by Ile and vice versa.

Antigenic equivalents may be formed by modifying reactive groups within a natural sequence or

modifying the N-terminal amino and/or C-terminal carboxyl group. Such equivalents include salts formed with acids and/or bases, particularly physiologically acceptable inorganic and organic acids and bases. Other equivalents include modified carboxyl and/or amino groups on the synthetic peptide to produce esters or amides, or amino acid protecting groups such as N-t-butoxycarbonyl. Preferred modifications are those which provide a more stable, active peptide which will be less prone to enzymatic degradation in vivo.

For use as a vaccine, the active agents of the present invention may be administered to the subject by any suitable means. Exemplary are by intramuscular injection, by subcutaneous injection, by intravenous injection, by intraperitoneal injection, by oral injection, and by nasal spray.

The amount of active agent administered will depend upon factors such as route of administration, species, and the use of booster administrations. In general, a dosage of about .1 to about 100 μg per pound subject body weight may be used, more particularly about 1 μg per pound.

Vaccine formulations of the present invention comprise the active agent in a pharmaceutically acceptable carrier. The active agent is included in the carrier in an amount effective to protect the subject being treated. Pharmaceutically acceptable carriers are preferably liquid, particularly aqueous, carriers, such as sodium phosphate buffered saline. The vaccine formulation may be stored in a sterile glass container sealed with a rubber stopper through which liquids may be injected and formulations withdrawn by syringe.

Vaccine formulations of the present invention may optionally contain one or more adjuvants. Any suitable adjuvant can be used, exemplary being aluminum hydroxide, aluminum phosphate, plant and animal oils, synthetic polymers and the like, with the amount of

adjuvant depending on the nature of the particular adjuvant employed. In addition, the vaccine formulations may also contain one or more stabilizer, exemplary being carbohydrates such as sorbitol, mannitol, starch, sucrose, dextrin, and glucose, proteins such as albumin or casein, and buffers such as alkaline metal phosphates and the like.

D. Infection of Cats with FIV Isolate $NCSU_1$.

Cats infected with FIV Isolate $NCSU_1$ are useful as a model system for the study of AIDS. Cats used for this purpose are preferably specific pathogen-free (SPF) cats, which are commercially available from sources such as Charles River Laboratories and Berkshire Laboratories. Infected cats are preferably maintained as a single colony of two or more cats, all infected with FIV isolate $NCSU_1$. The colony may be maintained in a single room with each cat housed in an appropriate cage, in accordance with standard practices for the maintenance of animals. Typically, a colony will consist of twenty to thirty cats, but this quantity will vary. Preferably, all members of the colony are SPF cats (i.e., free of pathogens other than FIV isolate $NCSU_1$).

SPF cats may be infected with NCSU Isolate $NCSU_1$ by any suitable means, such as by intraperitoneal, intravenous, or subcutaneous injection with a solution containing FIV Isolate $NCSU_1$. The solution may be blood from a previously infected cat, a blood fraction containing peripheral blood mononuclear cells from a previously infected cat, a pharmaceutically acceptable carrier such as saline solution containing FIV Isolate $NCSU_1$, etc.

Cats infected with FIV isolate $NCSU_1$ are particularly useful as a model system for AIDS because of the rapid inversion of the $CD4^+$:$CD8^+$ ratio caused by this virus. When used as a model system, the cat or cats infected with FIV isolate $NCSU_1$ is subjected to a treatment, which treatment is a candidate for use in

combating AIDS in human subjects, and the progress of the FIV infection cat or cats thereafter examined. A control group of FIV isolate $NCSU_1$ infected but untreated, or placebo treated, cats may be included for the purpose of comparison. A slowing in the progression of the disease in the cats indicates that the treatment may be useful for combating AIDS in humans. Typically, the candidate treatment will then be subjected to further screening procedures and toxicological testing to determine whether the treatment may be useful in treating humans afflicted with AIDS. The treatment to which the cats are subjected may be any treatment, such as the administration of candidate drugs (e.g., candidate antiretroviral compounds) or drug combinations, including small organic compounds (e.g., antiviral nucleosides such as AZT and DDI), peptides, or proteins, which may be administered orally or parenterally, or may involve treatments other than the administration of drugs such as a biological response modifier or a vaccine. The progress of the disease in the cats after treatment can be monitored by any suitable means, such as examination for inhibition of the deterioration of $CD4^+$ cell levels, declines in the circulating levels of the FIV GAG protein which corresponds to the p24 protein of HIV-1, the weight of the cat and its general appearance, etc.

E. Immunodeficient Mice containing Feline Tissue.

An advantage of using infected cats as a model for AIDS as described above is that the FIV virus is not infectious to humans. A disadvantage of this model is that cats are somewhat large animals. Alternate animal models are the SCID-hu mouse and the hu-PBL-SCID mouse infected with the human immunodeficiency virus type 1 (HIV-1). *See, e.g.*, J. McCune et al., *Science* **241**, 1632-39 (23 Sept. 1988); D. Mosier et al., *Nature* **335**, 256-59 (15 Sept. 1988). An advantage of the SCID-hu mouse as an animal model is its small size, but a serious disadvantage is that it carries the human AIDS virus.

Accordingly, there is a continuing need for small animal models of HIV-1 infection which do not employ a virus infectious to humans.

Disclosed herein is an immunodeficient mouse containing feline tissue, which feline tissue is capable of infection with feline immunodeficiency virus (FIV). The mouse is infected with FIV. Any isolate of FIV may be employed, with a preferred isolate being FIV isolate $NCSU_1$. Mice are infected with FIV and used as an animal model for human AIDS in essentially the same manner as cats as described above.

Any suitable immunodeficient mouse may be employed, such as SCID mice (e.g., the C.B.-17 *scid/scid* mouse) athymic mice such as the nude mouse, and mice which have been rendered immunodeficient by treatment with radiation. The mouse may be deficient in T lymphocytes function alone (e.g., athymic mice), but is preferably deficient in both T and B lymphocyte function.

The feline tissue which the immunodeficient mice contains preferably comprises one or more of the following: feline thymus tissue, feline lymph node tissue, feline liver cells, feline bone marrow cells, feline peripheral blood mononuclear cells such as peripheral blood lymphocytes and peripheral blood monocytes, and feline spleen cells. The feline tissue may be introduced into the mouse by any suitable means, such as intraperitoneal injection, intravenous injection, surgical implantation, and combinations thereof. Feline tissue may be introduced as organized tissues (e.g., thymus and lymph node) or as discrete cells. One example is an immunodeficient mouse having feline thymus tissue and/or lymph node tissue surgically implanted. Another example is an immunodeficient mouse into which peripheral blood mononuclear cells have been intraperitoneally injected.

F. Diagnostic Probes.

The FIV isolate $NCSU_1$ nucleotide sequence can be used to generate hybridization probes which specifically bind to FIV isolate $NCSU_1$ genetic material, or the genetic material of FIV isolates having the identifying characteristics of FIV isolate $NCSU_1$, to determine the presence of such FIV in cats. The hybridization probe may be selected so that it does not bind to other known FIV isolates, such as the Petaluma strain. The hybridization probes may be cDNA fragments or oligonucleotides, and may be labelled with a detectable group as discussed hereinbelow. Pairs of probes which will serve as PCR primers for the *axl* oncogene or a portion thereof may be used in accordance with the process described in U.S. Patents Nos. 4,683,202 and 4,683,195.

For example, an illustrative embodiment of the above probes comprises DNA sequences set forth in SEQ ID NO:6 or fragments thereof.

The term "labelled" is used herein to refer to the conjugating or covalent bonding of any suitable detectable group, including enzymes (e.g., horseradish peroxidase, β-glucuronidase, alkaline phosphatase, and β-D-galactosidase), fluorescent labels (e.g., fluorescein, luciferase), and radiolabels (e.g., ^{14}C, ^{131}I, ^{3}H, ^{32}P, and ^{35}S) to the compound being labelled. Techniques for labelling various compounds, including proteins, peptides, and antibodies, are well known. *See, e.g.*, Morrison, *Methods in Enzymology* **32b**, 103 (1974); Syvanen et al., *J. Biol. Chem.* **284**, 3762 (1973); Bolton and Hunter, *Biochem. J.* **133**, 529 (1973).

G. DNA Sequence and Genome Organization

Isolated DNA from the $NCSU^1$ provirus may be used to generate hybridization probes, which may be used in diagnostic assays as discussed above. Isolated DNA capable of expressing antigenic proteins or antigenic

fragments thereof may be used for producing such proteins, which are also useful in diagnostic assays.

An aspect of the present invention is oligonucleotide probes which selectively hybridize to DNA encoding a group antigen (gag) polypeptide (or an antigenic fragment thereof) of FIV $NCSU_1$ under stringent conditions, which probes do not bind to DNA encoding the group antigen (gag) polypeptide of the following known FIV strains under the same stringency conditions: FIV-Petaluma (U.S. Patent No. 5,037,753); FIV-PPR (Phillips *et al.*, *J. Virology*, **64**, 4605 (1990)); FIV-TM1 and FIV-TM2 (Miyazawa *et al.*, *Arch. Virology* **108**, 59 (1989)); FIV-UT113 (Verschoor *et al.*, *J. Cell. Biochem.*, **Suppl. 14D**, 143 (1990). Conditions which will permit other DNA coding for an FIV gag polypeptide to hybridize to the DNA of FIV $NCSU_1$ gag polypeptide can be determined in a routine manner. For example, hybridization may be carried out under conditions of reduced stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1% SDS at 60°C or even 70° C to DNA encoding the gag polypeptide of FIV $NCSU_1$ disclosed herein in a standard *in situ* hybridization assay. *See* J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2nd Ed. 1989)(Cold Spring Harbor Laboratory)).

In general, DNA which codes for FIV gag polypeptide or antigenic fragments thereof and which hybridizes to DNA encoding gag polypeptide (or antigenic fragments thereof) of FIV $NCSU_1$ disclosed herein will be at least 75% homologous, 80% homologous, or even 85% homologous or more with the DNA of the gag polypeptide (or antigenic fragments thereof) of FIV $NCSU_1$ disclosed herein. Further, DNA which codes for FIV gag polypeptide (or antigenic fragments thereof), or which codes for a gag polypeptide or antigenic fragment coded for by DNA which hybridizes to the DNA which codes for FIV $NCSU_1$ gag polypeptide or antigenic fragment thereof, but which

differ in codon sequence from these due to the degeneracy of the genetic code, are also an aspect of this invention. The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is well known in the literature. *See, e.g.*, U.S. Patent No. 4,757,006 to Toole et al. at Col. 2, Table 1.

A particular embodiment of the foregoing also disclosed herein is isolated DNA encoding a group antigen (gag) polypeptide, or an antigenic fragment thereof, of FIV $NCSU_1$, where the DNA is: **(a)** isolated DNA encoding group antigen (gag) polypeptide, or an antigenic fragment thereof, of FIV $NCSU_1$, **(b)** isolated DNA which hybridizes to isolated DNA of (a) above under stringent conditions and which encodes a feline immunodeficiency virus group antigen (gag) polypeptide or antigenic fragment thereof at least 75% homologous to isolated DNA of (a) above; or **(c)** isolated DNA differing from the isolated DNAs of (a) and (b) above in nucleotide sequence due to the degeneracy of the genetic code, and which encodes a feline immunodeficiency virus group antigen (gag) polypeptide or antigenic fragment thereof encoded by the isolated DNAs of (a) and (b), above.

An illustrative embodiment of the foregoing DNA which codes for FIV gag polypeptide (or antigenic fragments thereof) is DNA according to SEQ ID NO:6 or a fragment thereof.

Also disclosed herein are recombinant DNA sequences comprising vector DNA and a DNA encoding a group antigen (gag) polypeptide, or an antigenic fragment thereof, of FIV $NCSU_1$ (as given above).

The FIV provirus includes the structural genes for group-specific antigens (gag gene), envelope proteins (env gene) and reverse transcriptase (pol gene), as well as several short open reading frames similar to those of other lentiviruses. Omsted *et al.*, *Proc. Natl. Acad. Sci. USA*, **86**, 2448 (1989); Olmsted *et al.*, *Proc. Natl.*

Acad. Sci. USA, **86**, 8088 (1989). The gag gene of FIV has been reported to encode a polyprotein of about 450 amino acids, which is subjected to postranslational cleavage. Talbot *et al.*, *Proc. Natl. Acad. Sci. USA*, **86**, 5743 (1989); Phillips *et al.*, *J. Virology*, **64**, 4605 (1990). The gag gene and its predicted protein product has been reported to be highly conserved among isolates of FIV. Phillips *et al.*, *J. Virology*, **64**, 4605 (1990); Morikawa *et al.*, *Virology*, **183**, 288 (1991). FIV gag gene has been expressed in baculovirus vectors and assembled into virus-like particles. Morikawa *et al.*, *Virology*, **183**, 288 (1991).

Isolated and purified FIV $NCSU_1$ group antigen (gag) polypeptide or antigenic fragments thereof are also an aspect of the present invention. These polypeptides or fragments are coded for by: **(a)** isolated DNA which encodes group antigen (gag) polypeptide, or an antigenic fragment thereof, of FIV $NCSU_1$; **(b)** isolated DNA which hybridizes to isolated DNA of (a) above under stringent conditions and which encodes a FIV gag polypeptide or antigenic fragment thereof at least 75% homologous to isolated DNA of (a) above; or **(c)** isolated DNA differing from the isolated DNAs of (a) and (b) above in nucleotide sequence due to the degeneracy of the genetic code, and which encodes a FIV gag polypeptide or antigenic fragment thereof encoded by DNAs of (a) or (b), above. By antigenic polypeptide is meant a polypeptide which is able to raise (with the aid of an adjuvant if necessary) an antibody response in cats. The polypeptide may be a fragment or a polypeptide naturally occurring in FIV particles. The fragment may be from a naturally occurring polypeptide or produced by isolation or synthesis of a gene encoding a desired polypeptide and expression within an appropriate expression system.

An illustrative embodiment of the foregoing polypeptides is one having the amino acid sequence according to SEQ ID NO:7. Polypeptides of the present

invention include proteins homologous to, and having essentially the same biological properties as, the polypeptide of SEQ ID NO:7.

The present invention is explained in greater detail in the non-limiting Examples set forth below.

EXAMPLE 1

Animal Subjects

Nine adult, 3 to 5 year old, female, random source cats were used for this study. These cats had been in the laboratory animal care facility for two years prior to this study and their $CD4^+$:$CD8^+$ ratios determined several times during this period. Prior to infection, all animals were negative for feline leukemia virus (FeLV) by ELISA (TechAmerica, Omaha, NE) and FIV by Western blot using FIV antigen purified from CrFK cells chronically infected with FIV (obtained from Dr. John Black, American Biotech, Milton, TN). The cats had been vaccinated for feline panleukopenia, herpes, and calici viruses 10 months prior to this study. Four adult (1 year) specific pathogen free (SPF) cats were also used in one control group.

EXAMPLE 2

Isolation and Production of NCSU 1

Our original source of virus was from a cat (TOM) naturally infected with FIV as diagnosed by Western blot. TOM was negative for FeLV by ELISA. Peripheral blood mononuclear cells (PBMC) from TOM were demonstrated to carry FIV by Mg^{2+} dependent reverse transcriptase activity and by polymerase chain reaction (PCR) and Southern analysis using primers and probes to FIV LTR and gag sequences. Failure to generate Mn^{2+} dependent RT activity suggested that this cat was not infected with feline leukemia virus (FeLV) or feline syncytia forming virus (FeSFV). The $CD4^+$:$CD8^+$ ratio of TOM has been consistently below the 5th percentile reported for normal

random source and pet cats (0.57, determined from flow analysis of 39 adult random source and pet cats) and has ranged from 0.29 to 0.40 for over a year. *See* C. Novotney et al., *AIDS* **4:** 1213 (1990). PBMC from TOM were inoculated into two adult SPF cats (JJ and LOU) to provide a larger pool of cells for inoculum. Both SPF cats seroconverted by 2 months post infection (p.i.). By 6 months p.i., both cats had $CD4^+$:$CD8^+$ ratios below 1 (JJ=0.55, LOU=0.71). All three cats have remained positive for FIV by PCR/Southern and RT for a period of 6 months prior to and throughout the study reported here. FIV from TOM has been passaged in cultured feline PBMC for over a period of 6 months. We will refer to this virus throughout this text as the $NCSU_1$ isolate.

The $NCSU_1$ isolate (or "NCSU-1") was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 USA, in accordance with the provisions of the Budapest Treaty, on July 23, 1991, and has been assigned ATCC Number VR2333.

EXAMPLE 3

Infection of Subjects with NCSU 1

For infection of the cats in this study, peripheral blood was drawn from the three cats described above and the PBMC separated on a 43%/62% discontinuous Percoll gradient. *See* M. Tompkins et al., *Vet Immunol. Immunopathol.* **16:** 1 (1987). A fraction of the PBMC from each cat was set aside for PCR/Southern analysis and the remainder pooled, counted and incubated in culture for 48 hours with 10nM PMA. After 48 hours the cells were washed, counted and 2×10^6 PBMC inoculated intravenously into each of 6 adult random source cats. FIV infection was determined by the presence of antibody to the gag proteins (p15 and p26) by Western blot. *See, e.g.*, C. Novotney et al., *supra*. Four SPF cats obtained from Liberty Laboratories (Liberty Corner, NJ) were inoculated with 2×10^6 PMA-treated normal feline PBMC as mock-

infection controls and 3 random source cats were used as uninfected controls.

EXAMPLE 4

Seroconversion and Clinical Syndromes of Adult Cats Infected with NCSU 1

Six random source adult cats were inoculated with pooled PBMC from three cats infected with the $NCSU_1$ isolate of FIV in the manner described in Example 4 above. The donor cats were all seropositive for FIV at the time of bleeding by Western blot analysis, and their PBMC carried a relatively high burden of provirus as demonstrated by limiting dilution PCR and Southern analysis (**Fig. 1**). Plasma was collected prior to and at various weeks p.i. and tested for antibodies to FIV by Western blot in accordance with known procedures. *See, e.g.*, C. Novotney et al., *supra*. None of the cats demonstrated antibody to either of the FIV gag proteins, p15 and p26, prior to infection or 1 week p.i. By 2 weeks p.i., all 6 cats had developed antibody to either p15 or p26, and by 4 weeks p.i., and throughout the duration of the study, all 6 cats demonstrated antibody to both these proteins.

All 6 cats appeared clinically normal until 9 weeks p.i., when all the cats became depressed and lethargic. None of the cats, however, developed a fever, and only a mild lymphadenopathy was noted. By 15 weeks p.i., all cats appeared clinically normal and have remained so to date (9 months p.i.).

EXAMPLE 5

Co-Culture and Reverse Transcriptase Assay

The presence of FIV in the peripheral blood of cats infected as described in Example 4 was determined by reverse transcriptase (RT) assay of co-cultures in accordance with known procedures. *See, e.g.*, C. Novotney et al., *supra*. Briefly, PBMC from infected and normal cats were separated on Percoll and

incubated with 10 μg/ml Con A for 24 hours. Then 100 U/ml recombinant human IL-2 (Hoffman-LaRoche, Nutley, NJ) was added to the cultures. After 48 hours, 1 x 10^6 test PBMC were added to 2 x 10^6 normal PBMC and co-culture supernatants collected for assay for Mg^{2+}-dependent RT activity at 3-4 day intervals for 6 weeks.

The assay for RT activity was performed as described previously, *see* C. Novotney et al., *supra*, and is a modification of the procedure of Goff et al., *J. Virol.* **38:** 239 (1981). Ten μl of culture supernatant was added to 50 μl of an RT reaction mixture (0.5 μg/ml poly(A) oligo (dt) in 50 mM Tris [pH 7.8], 7.5mM KCL, 2 mM dithiothreitol, 5 mM $MgCl_2$, 0.05% Nonidet P-40, and 0.5 μCi [^{32}P] dTTP [ICN Biomedicals, Costa Mesa, CA]). After 2 hours at 37°C, 10 μl was spotted onto DE81 ion-exchange paper, dried, washed, and activity counted on a scintillation counter. Supernatant from FIV-infected CrFk cells was used as a positive control and supernatant from the target normal PBMC cultured alone was used as a negative control. All samples were run in quadruplicate. Results were converted to RT units which were calculated from the mean of quadruplicate samples of peak RT activity selected from sequential assays taken at 3-4 day intervals for 6 weeks after initiation of co-culture. RT units were calculated as follows:

$$\text{RT unit} = \frac{\text{Mean CPM of test supernatant - Mean CPM of negative control supernatant}}{\text{Mean CPM of negative control supernatant}}$$

EXAMPLE 6

Analysis of PBMC for FIV DNA by PCR Gene Amplification and Southern Analysis

For PCR quantification of cell-associated virus, 5 ml of blood from infected and control cats was collected in EDTA and separated on Percoll. Five ml of blood usually yielded about 1 X 10^7 PBMC. For limiting dilution PCR analysis, serial tenfold dilutions of PBMC, beginning with 1 x 10^6 and ending with 1 x 10^2, were made.

Each dilution was brought to a constant cell number of 2 x 10^6 cells with PBMC from a cat previously determined as FIV negative by PCR analysis.

Genomic DNA was collected following incubation in 500 microliters digestion buffer (100 mM NaCl, 10mM Tris Cl, pH8, 25mM EDTA, pH 8, 0.5% sodium dodecyl sulfate and 0.2 mg/ml Proteinase K) at 50°C for 18 hours. The DNA was purified by phenol extraction and ethanol precipitation, dried, and redissolved in 64 μl sterile distilled water.

Primers for the PCR reaction were selected from a published FIV sequence. *See* R. Olmsted et al., Molecular cloning of feline immunodeficiency virus, *Proc. Natl. Acad. Sci.* **86:** 2448 (1989). A 334 base pair fragment was amplified from the LTR region using primer U_{3-1} (GGATGAGTATTGGAACCCTGAA) (SEQ ID NO:1) and primer U_{5-1} (GATTCCGAGACCTCACAGGTAA) (SEQ ID NO:2). The PCR procedure was performed using the Gene Amp™ DNA amplification kit purchased from Perkin Elmer Cetus according to standard protocol. The entire 64 microliter DNA sample was used as template for each amplification.

After amplification, 10 microliters of the reaction product were run on a 1% Agarose gel, transferred to a nylon membrane (Biotrans™ membrane, ICN), and baked for 2 hours at 80°C. After prehybridization for 12 hours, the membrane was hybridized for 12 hours with an internally-located oligonucleotide probe (GGACTTTTGAGTTCTCCCTT) (SEQ ID NO:3) end-labeled on the 5′ end with ^{32}P-ATP (5′ DNA Terminus Labeling System™, BRL, Life Technologies, Inc.). The membrane was washed three times with 1 X SSC/0.1% SDS at room temperature for 15 minutes each and exposed to Kodak X-OMAT™ AR film between two intensifying screens (Fischer Biotech L Plus™) at -70°C. The film was processed after 2 and 12 hour exposures.

EXAMPLE 7

Cell Associated Viremia as Measured
by PCR/Southern Analysis and by RT Activity

Both HIV-1 and FIV establish a cell associated viremia that can be demonstrated by co-culture and RT activity. *See, e.g.*, M. McChesney and M. Oldstone, *supra*; N. Pedersen et al., *Science* **235**: 790 (1987). To determine how early after infection viremia was evident, lymphocytes from both the infected and uninfected cats were collected prior to and at 1, 2, 4, 6, and 9 weeks p.i., co-cultured with lymphocytes from normal cats, and the supernatants assayed for Mg^{2+}-dependent RT activity. **Table 1** lists the RT activity for each cat at the various sampling times prior to and post infection. Although co-cultured for six weeks, PBMC from all cats were negative for RT activity prior to infection. By 4 weeks p.i., high RT activity, ranging from 35 to 77 RT units, was detectable in 5 of the 6 infected cats. The 6th cat (LIL) had low (7 RT units) but detectible activity. All 6 cats showed RT activity by 6 weeks pi. In contrast, RT activity was not detected in the culture fluid of the uninfected control cats (TRX, HIY, HOO). Although all cats tested negative for FeLV infection prior to infection with FIV, we tested them again for possible Mn^{2+} dependent RT (indicative of FeLV and/or FeSFV infection) 6 weeks post FIV infection. All cats yielded high Mg^{2+}-dependent, but no Mn^{2+}-dependent RT activity. The results of one cat is shown in **Figure 2**.

TABLE 1. REVERSE TRANSCRIPTASE ACTIVITY IN PBMC CO-CULTURES FROM FIV-INFECTED AND NORMAL CATS

		FIV Reverse Transcriptase Units[1]					
		Weeks Post Infection					
Cat	Infection Status	0	1	2	4	6	9
TRX	Normal	0.2	0.4	1.6	3.0	0.5	0.3
HIY	Normal	0.7	0.0	0.2	2.1	0.8	0.6
HOO	Normal	0.1	0.3	0.8	0.2	0.9	0.2
PIX	Infected	1.3	41.5	6.1	51.0	49.3	89.6
MID	Infected	0.2	1.5	7.1	39.0	46.4	87.8
LIL	Infected	0.2	1.0	9.0	7.3	23.2	49.1
JIN	Infected	0.1	0.3	5.0	34.8	13.1	8.1
HEA	Infected	1.7	1.2	16.5	38.8	51.0	53.2
BUT	Infected	4.7	22.0	5.8	76.5	48.1	115.4

[1]RT units were calculated as given in Example 5 above.

At 4 weeks p.i., PBMC from 3 of the infected cats (PIX, MID, and LIL) and one control cat (TRX) were examined by PCR/Southern analysis for the presence of FIV provirus. All 3 infected cats were positive for FIV by PCR, while the normal cat was not. Thus although the PBMC from the cat LIL had very low RT activity at 4 weeks p.i. (7 units, Table 1), the PBMC were infected with FIV. All 6 infected cats were positive for FIV provirus at 4 months p.i. At 9 months (39 weeks) p.i., provirus was demonstrated by limiting dilution PCR/Southern analysis in as few as 10^2 - 10^3 PBMC in all 6 cats (data not shown), indicating a heavy virus burden.

EXAMPLE 8

Lymphocyte Subset Analysis

One week prior to and at various times after infection, blood was collected for a complete blood count (CBC) and flow cytometric analysis (FACS) of lymphocyte subpopulations using a panel of monoclonal antibodies developed in our laboratory (M. Tompkins et al., *Vet. Immunol. Immunopathol.* **26:** 305 (1990)). Cells were

purified and prepared for flow analysis as previously described. C. Novotney et al., *supra*. Briefly, cells were purified over Histopaque (Sigma Chemical Company, St. Louis, MO), density 1.083 and incubated at a concentration of 5 X 10^5 cells in 100 μl of monoclonal antibody (1.572 = Pan T; 3.357 = $CD8^+$; CAT30A = $CD4^+$; αIg = B cell) overnight at 4°C. The cells were then washed 3 times and incubated for 30 minutes at 4°C with a FITC-conjugated goat anti-mouse antibody that had been pre-absorbed with normal cat serum. The percent positively stained lymphocytes was determined by flow cytometric analysis using a Becton Dickinson FACScan. Absolute lymphocyte counts were performed on a Coulter counter by standard procedure.

EXAMPLE 9

Lymphocyte Subset Changes During Primary FIV Infection

To examine lymphocyte profiles during the early stage of FIV infection, PBMC were collected prior to infection and at various weeks p.i. and analyzed by flow cytometry for the distribution of B cells, T cells, $CD4^+$ T cells, and $CD8^+$ T cells. Samples were collected at the same time from three uninfected random source adult cats to monitor any changes associated with frequent sample collection. Figure 3A illustrates the $CD4^+$ and $CD8^+$ lymphocyte numbers and ratios of a representative cat (cat-MID). A lymphopenia developed at 2 weeks p.i. characterized by a profound decrease in both $CD4^+$ and $CD8^+$ lymphocyte populations. B cell numbers also decreased at this time (data not shown). The panlymphopenia was followed by a recovery of the $CD8^+$ and B cell populations at 4 weeks p.i. The $CD8^+$ cells continued to increase in number up to 9 weeks p.i., well beyond the preinfection level, where they leveled off and remained through the course of this study (39 weeks p.i.). In contrast, the $CD4^+$ population showed only a small recovery at 4 weeks p.i. and remained low throughout the 39 week study

period. This decrease in $CD4^+$ cells and increase in $CD8^+$ cells caused an early and prolonged inversion of the $CD4^+$:$CD8^+$ ratio in cat MID.

All of the six infected cats showed a similar decrease in $CD4^+$ numbers as cat MID. While all 6 cats demonstrated a recovery of $CD8^+$ cells at 4 weeks and beyond, not all cats showed increases beyond preinfection levels.

The average $CD4^+$ and $CD8^+$ numbers and ratios for all six cats are shown in **Figure 3B**. The pattern of response of the means of all 6 cats for $CD4^+$ and $CD8^+$ cell numbers and $CD4^+$:$CD8^+$ ratios is similar to the pattern shown by MID (**Fig. 3A**). After an initial lymphopenia, the $CD8^+$ cell numbers increase while the $CD4^+$ cell numbers do not, leading to a decline in the $CD4^+$:$CD8^+$ ratio. Statistical analysis of cell numbers regressed on time post infection demonstrated a significant relationship with time post infection for both $CD4^+$ ($p = 0.0005$) and $CD8^+$ ($p = 0.0271$) cells. In contrast, the $CD4^+$ and $CD8^+$ cell numbers in the uninfected controls remained steady throughout the study (**Fig. 4**) and were not significantly related to time p. i. Both cat populations developed a decreased number of circulating B cells during this study. Because this developed in the normal as well as the infected cats, it is likely related to repeat blood collection (data not shown).

The lymphocyte subset changes are dramatically illustrated by plotting the changes in the $CD4^+$:$CD8^+$ ratios of the FIV-infected cats. **Figure 5** compares the mean $CD4^+$:$CD8^+$ ratios of the control and FIV-infected cats. Although the mean $CD4^+$:$CD8^+$ ratio for the 6 cats prior to infection is below 1 (0.80 ± 0.26) and slightly below that of the three control cats (1.04 ± 0.06), the difference is not statistically significant, and the ratio is still within the normal range (0.57-1.81) determined from 39 adult random source cats. C. Novotney et al., *AIDS* **4:** 1213 (1990). The mean $CD4^+$:$CD8^+$ ratio of

the infected cats declined to levels below the 5th percentile for random source cats (0.57) by 4 weeks p.i. Similar to cell numbers, there is a significant relationship between $CD4^+$:$CD8^+$ ratio and time p.i. for the FIV-infected cats ($p < 0.0001$) but not the control cats. In addition, after 4 weeks p.i., there is a significant difference ($p < 0.0001$) in mean $CD4^+$:$CD8^+$ ratios between the FIV-infected cats and the control cats (Fig. 5).

To be sure that the cell changes seen in the FIV-infected cats were not a result of receiving foreign lymphocytes, 4 adult cats were inoculated with PMA-treated normal lymphocytes and their lymphocyte profiles examined at 2, 4, 10, and 16 weeks p.i. There were no changes in the lymphocyte distribution in any of the 4 cats as a result of inoculation with normal lymphocytes (**Fig. 6**). These results support the data suggesting that the lymphocyte changes seen in the FIV-infected cats are due to the virus infection.

In contrast to the studies reported herein, Pedersen et al., *J. Virol.* **64:** 598 (1990), reported no significant difference between normal cats and cats experimentally infected with FIV for less than a year. However, cats infected for a year or more were beginning to show inverted $CD4^+$:$CD8^+$ ratios. Moreover, Ackley et al., *J. Virol.* **64:** 5652 (1990), reported that experimental infection of SPF cats with FIV causes a reduction in the $CD4^+$:$CD8^+$ ratio only by 18 months or longer after infection. As was the case with some of the cats in our study, the $CD4^+$:$CD8^+$ inversion reported by Ackley et al. was due to a reduction in $CD4^+$ numbers as well as an increase in $CD8^+$ numbers. Our studies support those of Ackley et al. and suggest that FIV has a direct and profound effect on the immune system of the domestic cat.

We recently had the opportunity to compare our panel of monoclonal antibodies to feline lymphocyte subsets to those described and used by Ackley et al. (we

thank Dr.'s C. Ackley and M. Cooper for providing their monoclonal antibodies for feline CD4 (Fel7) and CD8 (FT2) markers for comparisons with our antibodies). We analyzed lymphocytes from both normal cats and cats infected with FIV, including the cats described herein, and found no differences in the percent positively staining cells with the two panels of antibodies.

EXAMPLE 10

Cloning of FIV Isolate $NCSU_1$

Feline peripheral blood mononuclear cells infected with FIV Isolate $NCSU_1$ are obtained as described above and a genomic DNA library constructed therefrom in accordance with standard procedures. *See* W. Strauss, Preparation of Genomic DNA from Mammalian Tissue, *in Current Protocols in Molecular Biology*, pp. 2.2.1-2.2.3 (F. Ausubel, R. Brent, R. Kingston, D. Moore, J. Seidman, J. Smith, and K. Struhl, eds. 1989)(New York: Greene Publishing Associates and Wiley-Interscience). The purified genomic DNA is partially digested with Sau3A I (J. Weiss, *in Current Protocols In Molecular Biology, supra* pp. 5.3.4-5.3.8) and separated on a 0.5% low-melt agarose gel. DNA fragments with a molecular weight of 14-20 Kb are purified in accordance with known procedures (*see* J. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, pp. 6.30-6.35 (2d Ed. 1989)(Cold Spring Harbor Laboratory Press)), ligated into EMBL-3 phage vector at the BAM HI cloning site, and packaged using the Packagene lambda gene packaging system.

Once the feline/$NCSU_1$ FIV provirus genomic DNA library is completed it is expressed and then screened for a full length genomic clone of the FIV provirus. Plaque lifts onto nitrocellulose membranes are screened via Southern hybridization with 5'LTR, 3'LTR, and GAG sequence specific DNA probes homologous to previous isolates of FIV to insure isolation of a full length clone.

The complete provirus clone is subcloned and purified for expression in feline host cells in accordance with either of two different procedures. In the first procedure, large quantities of proviral DNA are produced with the genomic clone, the DNA purified, and inserted into feline cells treated with calcium phosphate, DEAE-Dextran, or optionally with electroporation. *see* R. Kingston et al., *in Current Protocols in Molecular Biology, supra*, pp. 9.0.1-9.4.3. After transfection, the cells are treated to promote viral activity and thus produce an infectious clone. In the second method, the provirus from the EMBL-3 phage vector is cloned into a plasmid mammalian expression vector. A feline cell line is then transfected with the new provirus and vector, which provirus is then promoted with the expression vector's specific promoters.

EXAMPLE 11

Infection of SCID Mice with FIV

This example shows that when C.B.-17 *scid/scid* mice (SCID mice) are engrafted with sections of fetal feline thymus and/or lymph node, then given intraperitoneal injections of liver, bone marrow, peripheral blood lymphocytes, and/or spleen cells (SCID-fe mice), they are permissive for infection with feline immunodeficiency virus (FIV).

Fetal feline lymph node and thymus tissues are trimmed of fat and surgically implanted under the mammary fat pads of anesthetized C.B.-17 *scid/scid* mice. Immediately after implantation, each mouse is given a single intraperitoneal injection of a cell suspension comprised of finely minced feline liver, bone marrow, and spleen tissue, in physiological saline (approximately 10^8 cells in approximately 100-200 μl of solution). The exact proportion of liver, bone marrow, and spleen tissue may vary depending upon availability.

Two weeks after implantation, 27 SCID-fe mice prepared in essentially the same manner as described above were injected intraperitoneally with 7 x 10^6 $NCSU_1$ FIV-infected feline peripheral blood mononuclear cells (PBMC) and 2 SCID-fe mice were given 3 x 10^7 uninfected feline PBMC. Ten of these mice were given a dose of 125 mg/kg/day Retrovir® (azidothymidine, AZT) in the drinking water beginning 24 hours prior to virus challenge and continuing until the end of the study. Two weeks post-infection, the mice were sacrificed and implants were analyzed for FIV proviral DNA by PCR amplification of a 782 base pair segment of the gag open reading frame. Specificity was confirmed by hybridization to a radiolabeled internal oligonucleotide. The number of mice positive for FIV by PCR (summarized in Table 2 below) indicate a lower frequency of detection of FIV provirus in AZT-treated animals as compared to untreated.

TABLE 2: Detection of FIV provirus in AZT-treated and Untreated Mice.

	Untreated	AZT treated	Uninfected controls
Thymus implant	11/17 (65%)	2/10 (20%)	0/2 (0%)
Lymph node implant	11/17 (65%)	4/10 (40%)	0/2 (0%)
Both implants	8/17 (47%)	0/10 (0%)	0/2 (0%)

Hybridization intensities of FIV-positive samples in which equal amounts of DNA were amplified by PCR were compared to determine relative levels of provirus in each sample. Comparison of 5 untreated mice with 5 AZT-treated mice showed a significant reduction in provirus burden associated with AZT treatment. The stronger hybridization signal seen in the untreated animals suggests viral replication in the feline tissues. These data indicate that the FIV Infected SCID-fe mouse is a safe, realistic murine model for testing antiretroviral compounds.

EXAMPLE 12

Sequence of GAG gene of $NCSU_1$

The nucleotide sequence of the gag gene of $NCSU_1$ was determined in the following manner. Feline peripheral blood mononuclear cells infected with FIV Isolate $NCSU_1$ were obtained as described above (*see* Examples 3-6) and a genomic DNA library constructed therefrom in accordance with standard procedures. *See* W. Strauss, Preparation of Genomic DNA from Mammalian Tissue, In *Current Protocols in Molecular Biology*, pp. 2.21-2.23, F. Ausubel *et al.* (Eds.), New York: Greene Publishing Associates and Wiley-Interscience (1989). The gag gene of $NCSU_1$ was amplified by PCR using primers complementary to nucleotides 610-631 (SEQ ID NO:4) and 2026-2005 (SEQ ID NO:5) of FIV strain PPR (Phillips, *et al.*, *J. Virol.* **64**, 4605 (1990); GenBank accession no. m36968):

GAGAGACTCT ACAGCAACAT GG (SEQ. ID NO.:4)

AGACCGGAGA AAAGATTACT AC (SEQ. ID NO.:5)

The primers also contained restriction enzyme sites on the 5′ ends to facilitate subcloning into appropriate plasmid vectors. Plasmid PSL 1190 (Pharmacia LKB Biotechnology, Piscataway, New Jersey) was used, and the Xho I - BGL II site was used. *See* Brosius J. *et al.*, DNA, **8**, 759 (1989). Both strands of the cloned gag gene fragment of $NCSU_1$ were then sequenced by the dideoxynucleotide chain-termination method (Singer F, *et al*, Proc. Natl. Acad. Sci. USA, **74**, 5463 (1977)) using Sequenase version 2.0 and T7 DNA polymerase (U.S. Biochemical, Cleveland, Ohio; used as described in manufacturer's instructions for double-stranded DNA). Computer analyses of the nucleotide and predicted amino acid sequences were performed with MacVector (International Biotechnologies Inc., New Haven, Conn.). Open reading frames (orfs) were identified by Fickett's method, which employs a TESTCODE algorithm and statistical parameters for predicting protein coding

regions in DNA sequences. Fickett, *Nucl. Acids Res.* **10**, 5303 (1982). Orfs defined by Fickett's method as having a coding probability above 0.92 were accepted as potential protein coding regions.

Two orfs of 1350 and 150 base pairs were identified. The 1350 bp orf appeared to correspond to the gag orf by sequence comparisons to other FIV strains: FIV PPR; FIV-CG (GenBank accession number M25729); FIV-14 (GenBank accession number M25381); GenBank file FIV-Dixon; GenBank file FIV IMMDEF A; GenBank file FIV IMMDEF B; GenBank file FIV Z1; and GenBank file FIV GVEPX. The gag orf product is predicted to be 450 amino acids in length with a molecular mass of 49 kilodaltons. The predicted sites of gag polypeptide cleavage are before Pro-136 and after Leu-362, which results in gag polypeptides of 15, 25, and 10 kilodaltons. These predictions are the same as that observed for FIV strains Petaluma and PPR. Talbott *et al.*, Proc. Natl. Acad. Sci. USA, **86**, 5743 (1989); Phillips *et al.*, J. Virol. **64**, 4605 (1990). The nucleotide and predicted amino acid sequences for the $NCSU_1$ gag gene are shown in SEQ ID NO:6 and SEQ ID NO:7, respectively.

A 150 base pair orf (designated ORF 5) was found within the cloned gag gene fragment of $NCSU_1$, at nucleotide 663-812. This small orf is not present in reported DNA sequences of other FIV strains, as listed above. Four small orfs have been identified in the genome of FIV 14 and are located within the pol-env intergenic region, env, and sequence 3' to env (Olmsted *et al.*, *Proc. Natl. Acad. Sci. USA*, **86**, 8088 1989). Small orfs are a characteristic of other lentiviral genomes and are essential for the regulation of viral gene expression and replication. *See* Peterlin and Luciw, *AIDS*, **2, Suppl 1**, S29 (1988).

The ORF 5 gene product is predicted to be 50 amino acids in length, have a molecular mass of 5.9KDa and an isoelectric point of 7.32, be leucine rich (20%)

and basic (18% lysine + histidine + arginine). Attempts to align the deduced amino acid sequence of ORF 5 with analogous sequences from other lentiviruses (HIV, SIV, visna virus, and equine infectious anemia virus) were unsuccessful. The nucleotide and deduced amino acid sequences of $NCSU_1$ ORF 5 are shown in SEQ ID NO:8 and SEQ ID NO:9, respectively. ORF 5 is one nucleotide out of frame with the gag orf.

The ORF 5 gene product is predicted to have a helix-turn-helix structural motif, spanning amino acids 18-41, which is found in some DNA-binding proteins. A potential RNA splice-acceptor site is located near the start of ORF 5, indicating that this orf may serve as an exon for a spliced transcript. This data suggests that the ORF 5 gene product functions in the regulation of viral gene expression or replication, and distinguished $NCSU_1$ from other FIV strains.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Tompkins, Wayne A.
Tompkins, Mary B.

(ii) TITLE OF INVENTION: Feline Immunodeficiency Virus Isolate NCSU-1

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Bell, Seltzer, Park & Gibson
(B) STREET: Post Office Drawer 34009
(C) CITY: Charlotte
(D) STATE: North Carolina
(E) COUNTRY: U.S.A.
(F) ZIP: 28234

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US to be assigned
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Sibley, Kenneth D.
(B) REGISTRATION NUMBER: 31,665
(C) REFERENCE/DOCKET NUMBER: 5051-155A

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (919) 881-3140
(B) TELEFAX: (919) 881-3175

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATGAGTAT TGGAACCCTG AA 22

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATTCCGAGA CCTCACAGGT AA 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGACTTTTGA GTTCTCCCTT 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTCTCTGAGA TGTCGTTGTA CC 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCTGGCCTCA TTTCTAATGA TG 22

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1418 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 23..1372

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GTAGGAGAGA TTCTACAGCA AC ATG GGG AAT GGA CAG GGG CGA GAT TGG AAA      52
                         Met Gly Asn Gly Gln Gly Arg Asp Trp Lys
                           1               5                  10

ATG GCC ATT AAG AGA TGT AGT AAT GCT GCT GTA GGA GTA GGG GGG AAG      100
Met Ala Ile Lys Arg Cys Ser Asn Ala Ala Val Gly Val Gly Gly Lys
                  15                  20                  25

AGT AAA AAA TTT GGG GAA GGG AAT TTC AGA TGG GCC ATT AGA ATG GCT      148
Ser Lys Lys Phe Gly Glu Gly Asn Phe Arg Trp Ala Ile Arg Met Ala
              30                  35                  40

AAT GTA TCT ACA GGA CGA GAA CCT GGT GAT ATA CCA GAG ACT TTA GAT      196
Asn Val Ser Thr Gly Arg Glu Pro Gly Asp Ile Pro Glu Thr Leu Asp
          45                  50                  55

CAA CTA AGG TTG GTT ATT TGC GAT TTA CAA GAA AGA AGA AAA AAA TTT      244
Gln Leu Arg Leu Val Ile Cys Asp Leu Gln Glu Arg Arg Lys Lys Phe
      60                  65                  70
```

GGA TCT TGC AAA GAA ATT GAT AAG GCA ATT GTT ACA TTA AAA GTC TTT 292
Gly Ser Cys Lys Glu Ile Asp Lys Ala Ile Val Thr Leu Lys Val Phe
75 80 85 90

GCG GCA GTA GGA CTT TTA AAT ATG ACA GTG TCT TCT GCT GCT GCA GCT 340
Ala Ala Val Gly Leu Leu Asn Met Thr Val Ser Ser Ala Ala Ala Ala
95 100 105

GAA AAT ATG TTC ACT CAG ATG GGA TTA GAC ACT AGA CCA TCT ATG AAA 388
Glu Asn Met Phe Thr Gln Met Gly Leu Asp Thr Arg Pro Ser Met Lys
110 115 120

GAA GCA GGA GGA AAA GAG GAA GGC CCT CCA CAG GCA TTT CCT ATT CAA 436
Glu Ala Gly Gly Lys Glu Glu Gly Pro Pro Gln Ala Phe Pro Ile Gln
125 130 135

ACA GTA AAT GGA GTA CCA CAA TAT GTA GCA CTT GAC CCA AAA ATG GTG 484
Thr Val Asn Gly Val Pro Gln Tyr Val Ala Leu Asp Pro Lys Met Val
140 145 150

TCC ATT TTT ATG GAA AAG GCA AGA GAA GGA TTA GGA GGT GAG GAA GTT 532
Ser Ile Phe Met Glu Lys Ala Arg Glu Gly Leu Gly Gly Glu Glu Val
155 160 165 170

CAG CTA TGG TTC ACT GCC TTC TCT GCA AAT TTA ACA CCT ACT GAC ATG 580
Gln Leu Trp Phe Thr Ala Phe Ser Ala Asn Leu Thr Pro Thr Asp Met
175 180 185

GCC ACA TTA ATA ATG GCC GCA CCA GGG TGC GCT GCA GAT AAA GAA ATA 628
Ala Thr Leu Ile Met Ala Ala Pro Gly Cys Ala Ala Asp Lys Glu Ile
190 195 200

TTG GAT GAA AGC TTA AAG CAA CTT ACT GCA GGA TAT GAT CGT ACA CAT 676
Leu Asp Glu Ser Leu Lys Gln Leu Thr Ala Gly Tyr Asp Arg Thr His
205 210 215

CCC CCT GAT GCT CCC AGA CCA TTA CCC TAT TTT ACT GCA GCA GAA ATT 724
Pro Pro Asp Ala Pro Arg Pro Leu Pro Tyr Phe Thr Ala Ala Glu Ile
220 225 230

ATG GGT ATT GGA TTT ACT CAA GAA CAA CAA GCA GAA GCA AGA TTT GCA 772
Met Gly Ile Gly Phe Thr Gln Glu Gln Gln Ala Glu Ala Arg Phe Ala
235 240 245 250

CCA GCT AGG ATG CAG TGT AGA GCA TGG TAT CTC GAG GGA CTA GGA AAA 820
Pro Ala Arg Met Gln Cys Arg Ala Trp Tyr Leu Glu Gly Leu Gly Lys
255 260 265

TTG GGC GCC ATA AAA GCT AAG TCT CCT CGA GCT GTG CAG TTA AGA CAA 868
Leu Gly Ala Ile Lys Ala Lys Ser Pro Arg Ala Val Gln Leu Arg Gln
270 275 280

GGA GCT AAG GAA GAT TAT TCA TCC TTT ATT GAC AGA TTG TTT GCC CAA 916
Gly Ala Lys Glu Asp Tyr Ser Ser Phe Ile Asp Arg Leu Phe Ala Gln
285 290 295

```
ATA GAT CAA GAA CAA AAT ACA GCT GAA GTT AAG TTA TAT TTA AAA CAG          964
Ile Asp Gln Glu Gln Asn Thr Ala Glu Val Lys Leu Tyr Leu Lys Gln
    300                 305                 310

TCA TTA AGC ATG GCT AAT GCT AAT GCA GAA TGT AAA AAG CCA ATG ACC         1012
Ser Leu Ser Met Ala Asn Ala Asn Ala Glu Cys Lys Lys Pro Met Thr
315                 320                 325                 330

CAC CTT AAG CCA GAA AGT ACC CTA GAA GAA AAG TTG AGA GCT TGT CAA         1060
His Leu Lys Pro Glu Ser Thr Leu Glu Glu Lys Leu Arg Ala Cys Gln
                335                 340                 345

GAA ATA GGC TCA CCA GGA TAT AAA ATG CAA CTC TTG GCA GAA GCT CTT         1108
Glu Ile Gly Ser Pro Gly Tyr Lys Met Gln Leu Leu Ala Glu Ala Leu
            350                 355                 360

ACA AAA GTT CAA GTA GTG CAA TCA AAA GGA TCA GGA CCA GTG TGT TTT         1156
Thr Lys Val Gln Val Val Gln Ser Lys Gly Ser Gly Pro Val Cys Phe
        365                 370                 375

AAT TGT AAA AAA CCA GGA CAT CTA GCA AGA CAA TGT AGA GAA GTG AGA         1204
Asn Cys Lys Lys Pro Gly His Leu Ala Arg Gln Cys Arg Glu Val Arg
    380                 385                 390

AAA TGT AAT AAA TGT GGA AAA CCT GGT CAT GTA GCT GCC AAA TGT TGG         1252
Lys Cys Asn Lys Cys Gly Lys Pro Gly His Val Ala Ala Lys Cys Trp
395                 400                 405                 410

CAA GGA AAT AGA AAG AAT TCG GGA AAC TGG AAG GCG GGG CGA GCT GCA         1300
Gln Gly Asn Arg Lys Asn Ser Gly Asn Trp Lys Ala Gly Arg Ala Ala
                415                 420                 425

GCC CCA GTG AAT CAA GTG CAG CAA GCA GTA ATG CCA TCT GCA CCT CCA         1348
Ala Pro Val Asn Gln Val Gln Gln Ala Val Met Pro Ser Ala Pro Pro
            430                 435                 440

ATG GAG GAG AAA CTA TTG GAT TTA TAAATTATAA TAGAGTAGGT ACTACTACAA        1402
Met Glu Glu Lys Leu Leu Asp Leu
        445                 450

CATTAGAAAA GAGGCC                                                       1418
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 450 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Gly Asn Gly Gln Gly Arg Asp Trp Lys Met Ala Ile Lys Arg Cys
1 5 10 15

Ser Asn Ala Ala Val Gly Val Gly Gly Lys Ser Lys Lys Phe Gly Glu
20 25 30

Gly Asn Phe Arg Trp Ala Ile Arg Met Ala Asn Val Ser Thr Gly Arg
35 40 45

Glu Pro Gly Asp Ile Pro Glu Thr Leu Asp Gln Leu Arg Leu Val Ile
50 55 60

Cys Asp Leu Gln Glu Arg Arg Lys Lys Phe Gly Ser Cys Lys Glu Ile
65 70 75 80

Asp Lys Ala Ile Val Thr Leu Lys Val Phe Ala Ala Val Gly Leu Leu
85 90 95

Asn Met Thr Val Ser Ser Ala Ala Ala Ala Glu Asn Met Phe Thr Gln
100 105 110

Met Gly Leu Asp Thr Arg Pro Ser Met Lys Glu Ala Gly Gly Lys Glu
115 120 125

Glu Gly Pro Pro Gln Ala Phe Pro Ile Gln Thr Val Asn Gly Val Pro
130 135 140

Gln Tyr Val Ala Leu Asp Pro Lys Met Val Ser Ile Phe Met Glu Lys
145 150 155 160

Ala Arg Glu Gly Leu Gly Gly Glu Glu Val Gln Leu Trp Phe Thr Ala
165 170 175

Phe Ser Ala Asn Leu Thr Pro Thr Asp Met Ala Thr Leu Ile Met Ala
180 185 190

Ala Pro Gly Cys Ala Ala Asp Lys Glu Ile Leu Asp Glu Ser Leu Lys
195 200 205

Gln Leu Thr Ala Gly Tyr Asp Arg Thr His Pro Pro Asp Ala Pro Arg
210 215 220

Pro Leu Pro Tyr Phe Thr Ala Ala Glu Ile Met Gly Ile Gly Phe Thr
225 230 235 240

Gln Glu Gln Gln Ala Glu Ala Arg Phe Ala Pro Ala Arg Met Gln Cys
245 250 255

Arg Ala Trp Tyr Leu Glu Gly Leu Gly Lys Leu Gly Ala Ile Lys Ala
260 265 270

Lys Ser Pro Arg Ala Val Gln Leu Arg Gln Gly Ala Lys Glu Asp Tyr
275 280 285

Ser Ser Phe Ile Asp Arg Leu Phe Ala Gln Ile Asp Gln Glu Gln Asn
290 295 300

Thr Ala Glu Val Lys Leu Tyr Leu Lys Gln Ser Leu Ser Met Ala Asn
305 310 315 320

Ala Asn Ala Glu Cys Lys Lys Pro Met Thr His Leu Lys Pro Glu Ser
325 330 335

Thr Leu Glu Glu Lys Leu Arg Ala Cys Gln Glu Ile Gly Ser Pro Gly
340 345 350

Tyr Lys Met Gln Leu Leu Ala Glu Ala Leu Thr Lys Val Gln Val Val
355 360 365

Gln Ser Lys Gly Ser Gly Pro Val Cys Phe Asn Cys Lys Lys Pro Gly
370 375 380

His Leu Ala Arg Gln Cys Arg Glu Val Arg Lys Cys Asn Lys Cys Gly
385 390 395 400

Lys Pro Gly His Val Ala Ala Lys Cys Trp Gln Gly Asn Arg Lys Asn
405 410 415

Ser Gly Asn Trp Lys Ala Gly Arg Ala Ala Ala Pro Val Asn Gln Val
420 425 430

Gln Gln Ala Val Met Pro Ser Ala Pro Pro Met Glu Glu Lys Leu Leu
435 440 445

Asp Leu
450

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..150

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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ATG ATC GTA CAC ATC CCC CTG ATG CTC CCA GAC CAT TAC CCT ATT TTA        48
Met Ile Val His Ile Pro Leu Met Leu Pro Asp His Tyr Pro Ile Leu
 1               5                  10                  15

CTG CAG CAG AAA TTA TGG GTA TTG GAT TTA CTC AAG AAC AAC AAG CAG        96
Leu Gln Gln Lys Leu Trp Val Leu Asp Leu Leu Lys Asn Asn Lys Gln
            20                  25                  30

AAG CAA GAT TTG CAC CAG CTA GGA TGC AGT GTA GAG CAT GGT ATC TCG       144
Lys Gln Asp Leu His Gln Leu Gly Cys Ser Val Glu His Gly Ile Ser
        35                  40                  45

AGG GAC                                                              150
Arg Asp
    50
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Ile Val His Ile Pro Leu Met Leu Pro Asp His Tyr Pro Ile Leu
 1               5                  10                  15

Leu Gln Gln Lys Leu Trp Val Leu Asp Leu Leu Lys Asn Asn Lys Gln
            20                  25                  30

Lys Gln Asp Leu His Gln Leu Gly Cys Ser Val Glu His Gly Ile Ser
        35                  40                  45

Arg Asp
    50
```

THAT WHICH IS CLAIMED IS:

1. An isolated feline immunodeficiency virus (FIV) having the identifying characteristics of FIV isolate $NCSU_1$.

2. A biologically pure culture of host cells containing the feline immunodeficiency virus of claim 1.

3. Isolated DNA coding for a feline immunodeficiency virus of claim 1 or an antigenic fragment thereof.

4. A vector comprising DNA coding for a feline immunodeficiency virus of claim 1 or an antigenic fragment thereof.

5. A vector according to claim 4, wherein said vector comprises bacteriophage lambda.

6. A host cell containing and capable of expressing a vector according to claim 4.

7. A host cell according to claim 6, wherein said host cell comprises *Escherichia coli*.

8. A host cell according to claim 6, wherein said host cell comprises a yeast cell.

9. A host cell according to claim 6, wherein said host cell comprises a mammalian host cell.

10. A specific pathogen free (SPF) cat infected with feline immunodeficiency virus isolate $NCSU_1$.

11. A colony of SPF cats according to claim 10.

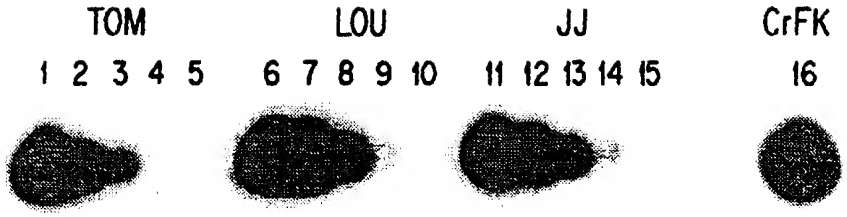

FIG. 1

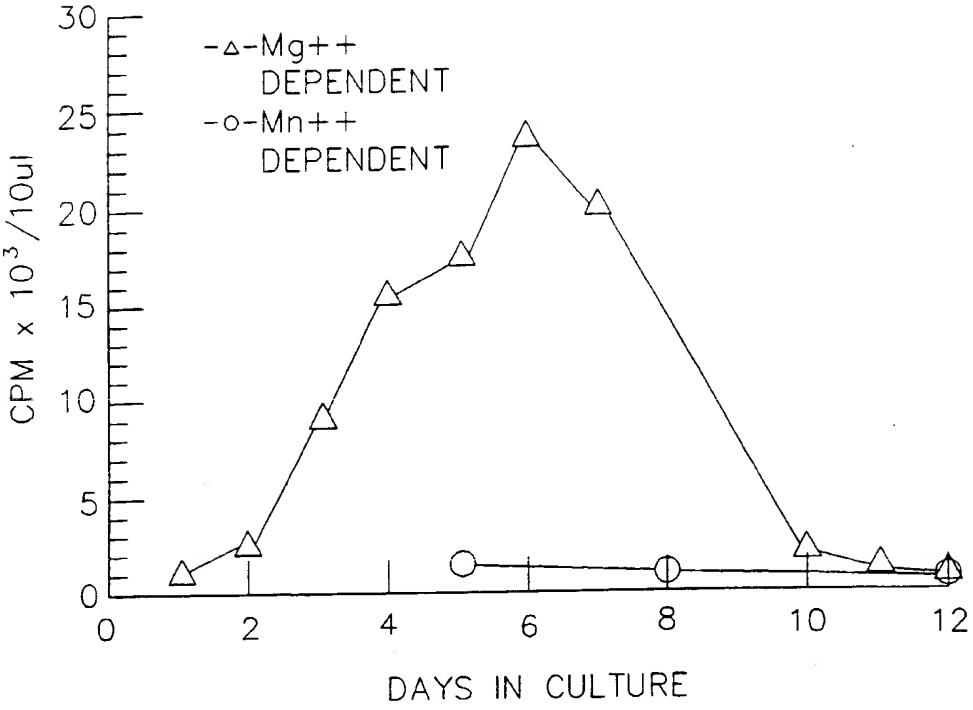
30
25
20
15
10
5
0
CPM x 10^3/10ul
-△-Mg++
DEPENDENT
-o-Mn++
DEPENDENT
0
2
4
6
8
10
12
DAYS IN CULTURE

FIG. 2.

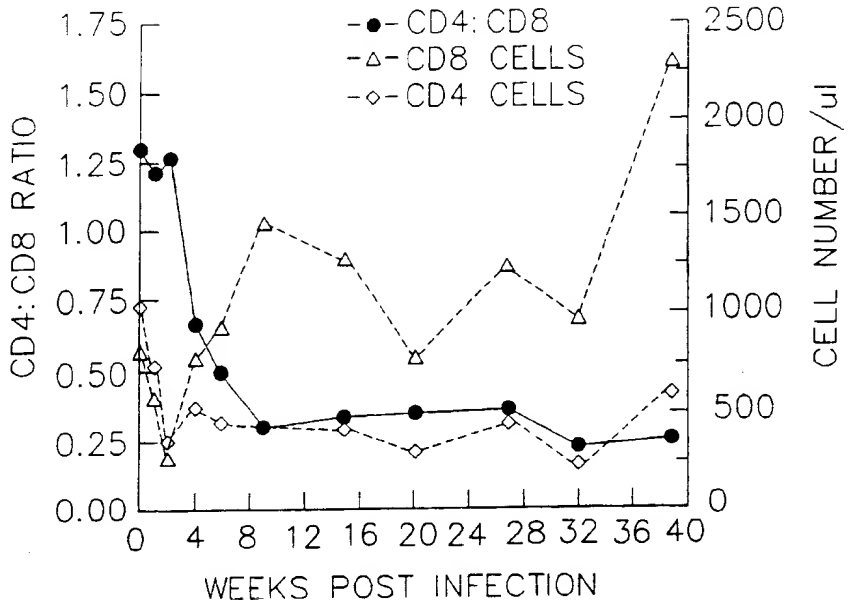
CD4:CD8
CD8 CELLS
CD4 CELLS
CD4:CD8 RATIO
CELL NUMBER/ul
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
2500
2000
1500
1000
500
0
0 4 8 12 16 20 24 28 32 36 40
WEEKS POST INFECTION

FIG. 3A.

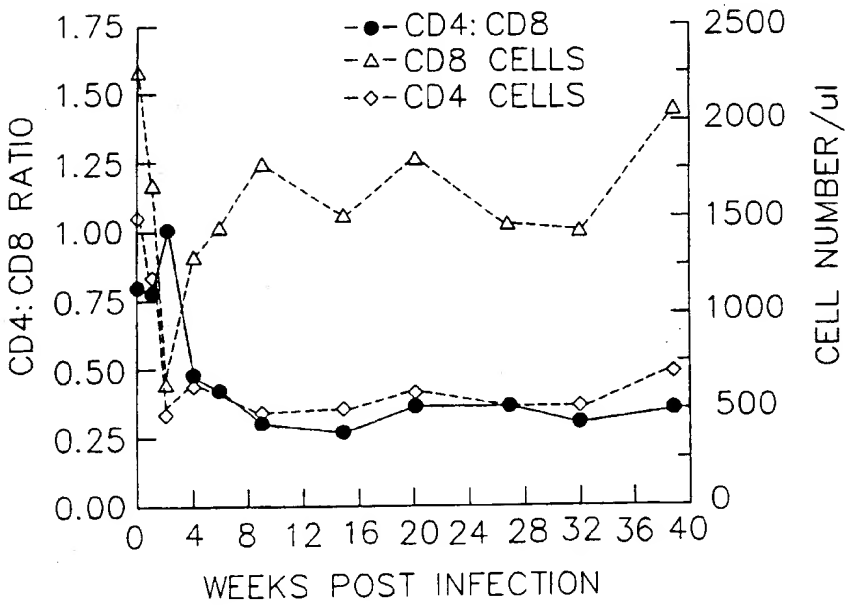
CD4:CD8
CD8 CELLS
CD4 CELLS
CD4:CD8 RATIO
CELL NUMBER/ul
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
2500
2000
1500
1000
500
0
0 4 8 12 16 20 24 28 32 36 40
WEEKS POST INFECTION

FIG. 3B.

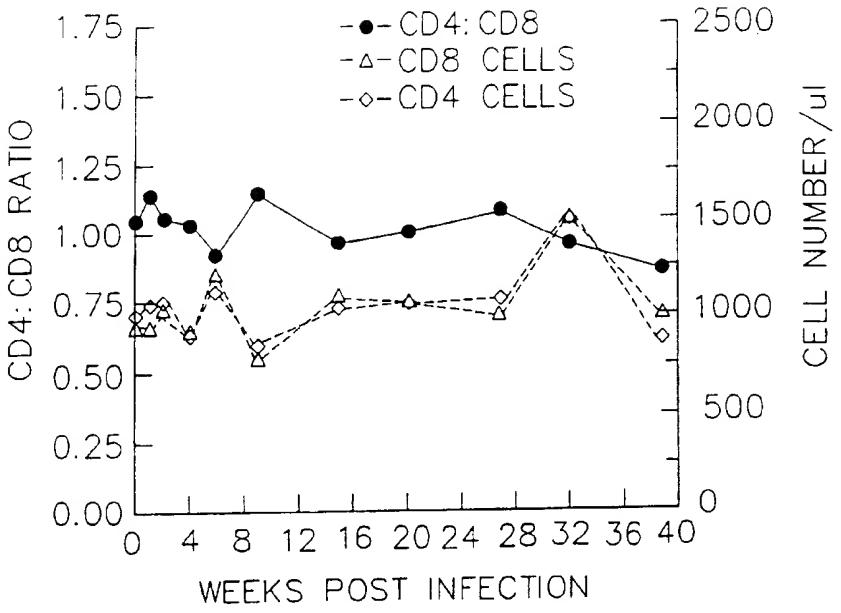
-●- CD4: CD8
-△- CD8 CELLS
-◇- CD4 CELLS
CD4: CD8 RATIO
CELL NUMBER/ul
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
2500
2000
1500
1000
500
0
0 4 8 12 16 20 24 28 32 36 40
WEEKS POST INFECTION

FIG. 4.

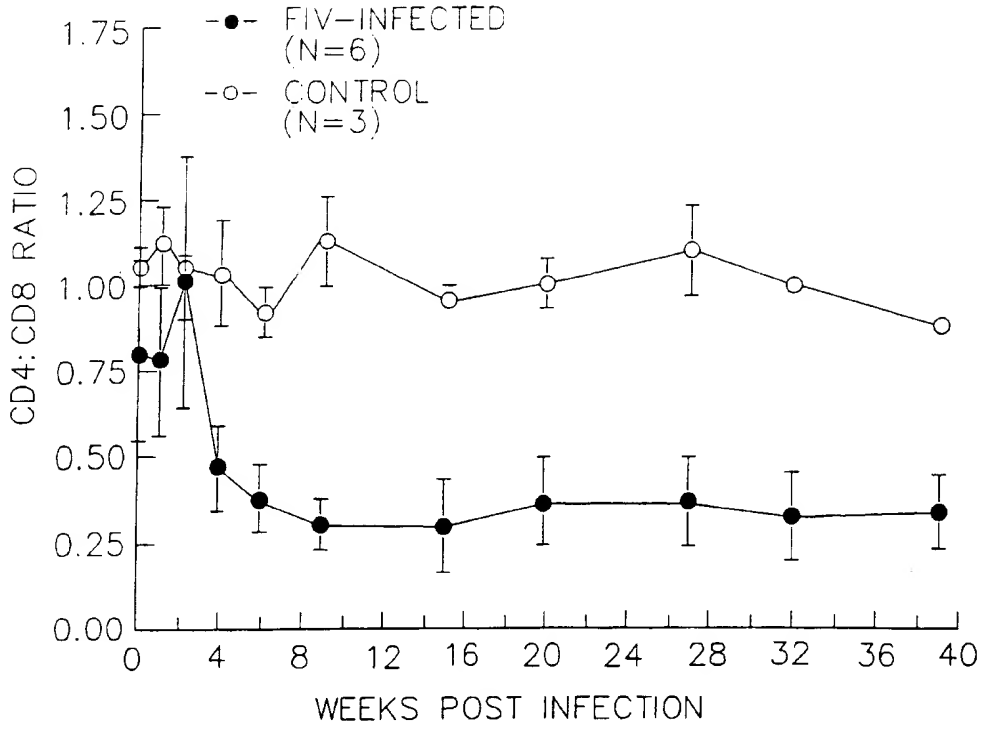
-●- FIV-INFECTED (N=6)
-o- CONTROL (N=3)
CD4:CD8 RATIO
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
4
8
12
16
20
24
28
32
36
40
WEEKS POST INFECTION

FIG. 5.

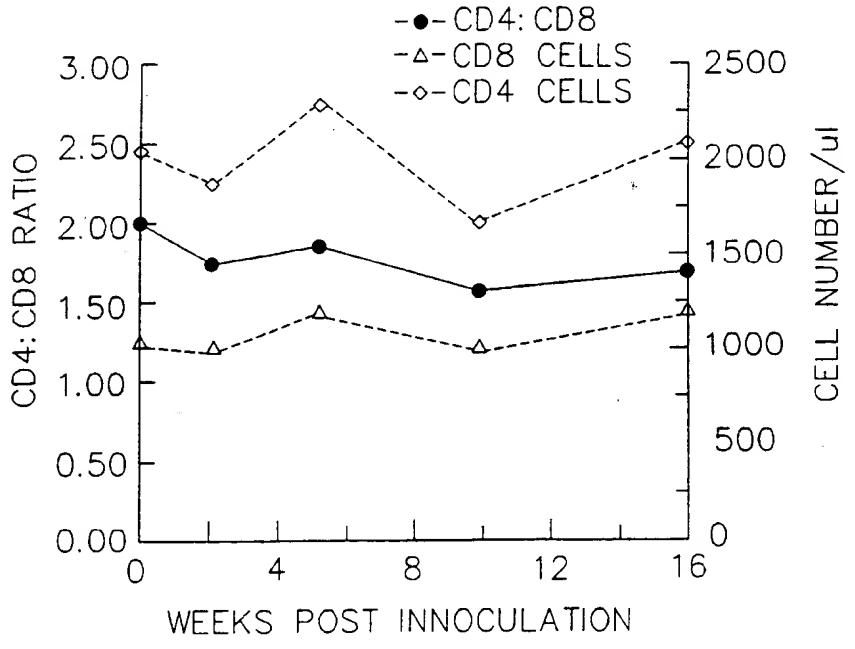
-●-CD4:CD8
-△-CD8 CELLS
-◇-CD4 CELLS
CD4:CD8 RATIO
3.00
2.50
2.00
1.50
1.00
0.50
0.00
2500
2000
1500
1000
500
0
CELL NUMBER/ul
0
4
8
12
16
WEEKS POST INNOCULATION

FIG. 6.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/48, 1/21, 1/19, 5/10, 7/00
A01K 67/027, C07K 14/155

A3

(11) International Publication Number: **WO 95/05460**

(43) International Publication Date: 23 February 1995 (23.02.95)

(21) International Application Number: PCT/US94/08364

(22) International Filing Date: 25 July 1994 (25.07.94)

(30) Priority Data:
105,710 12 August 1993 (12.08.93) US

(60) Parent Application or Grant
(63) Related by Continuation
US 105,710 (CON)
Filed on 12 August 1993 (12.08.93)

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(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: TOMPKINS, Wayne, A., F. [US/US]; 6817 Branton Drive, Apex, North Carolina 27502 (US). TOMPKINS, Mary, B. [US/US]; 6817 Branton Drive, Apex, NC 27502 (US).

(74) Agents: SIBLEY, Kenneth, D. et al.; Bell, Seltzer, Park & Gibson, P.O. Drawer 34009, Charlotte, NC 28234 (US).

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(57) Abstract

Disclosed is an isolated and purified feline immunodeficiency virus (FIV) culture having the identifying characteristics of FIV isolate $NCSU_1$. A biologically pure culture of host cells containing an FIV having the identifying characteristics of FIV isolate $NCSU_1$ is also disclosed, along with isolated and purified DNA coding for (a) an FIV having the identifying characteristics of FIV isolate $NCSU_1$, or (b) an antigenic fragment of an FIV having the identifying characteristics of FIV isolate $NCSU_1$. Various vaccine formulations containing active agents derived from the foregoing FIV virus, DNA encoding the virus, and DNA encoding antigenic fragments of the virus are also disclosed herein. Also disclosed are immunodeficient mice containing feline tissue, which feline tissue is capable of infection with a feline immunodeficiency virus such as (but not limited to) FIV isolate $NCSU_1$.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 94/08364

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/48 C12N1/21 C12N1/19 C12N5/10 C12N7/00
A01K67/027 C07K14/155

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION, vol.199, no.10, 15 November 1991 pages 1311 - 1315 MARY B. TOMPKINS ET AL. 'Early events in the immunopathogenesis of feline retrovirus infection' see page 1312, right column, paragraph 4 --- -/--	1,2,10, 11

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 February 1995	15.02.95

Name and mailing address of the ISA	Authorized officer
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PCT/US 94/08364

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol.86, no.15, August 1989, WASHINGTON US pages 5743 - 5747 RANDY L. TALBOTT ET AL. 'Nucleotide sequence and genomic organization of feline immunodeficiency virus' cited in the application see abstract see page 5743, left column, paragraph 2 - page 5745, right column, paragraph 1 ---	1-4,6,9
A	JOURNAL OF CELLULAR BIOCHEMISTRY. KEYSTONE SYMPOSIA ON MOLECULAR & CELLULAR BIOLOGY 16E, 1992, page 55 see abstract no. Q 345 ---	1,2
P,X	THE AMERICAN JOURNAL OF PATHOLOGY, vol.143, no.5, November 1993 pages 1486 - 1497 MICHAEL G. DAVIDSON ET AL. 'Feline immunodeficiency virus predisposes cats to acute generalized toxoplasmosis' see page 1487, right column, paragraph 1 - page 1488, left column, paragraph 1 see page 1489, left column, paragraph 4 ---	1,2,10, 11
P,X	JOURNAL OF VIROLOGY, vol.67, no.9, September 1993 pages 5175 - 5186 ROBERT V. ENGLISH ET AL. 'In vivo lymphocyte tropism of feline immunodeficiency virus' see page 5175, left column, paragraph 2 - page 5176, left column, paragraph 3 see page 5177, left column, last paragraph - right column, paragraph 1 -----	1,2,10, 11

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